Leptin Levels in Lymph Node Aspiration Biopsy is a Predictor of Smoking Tendencies: A Pilot Study

Fares Qeadan (fares.qeadan@utah.edu)
University of Utah https://orcid.org/0000-0002-3376-220X
Rosstin Ahmadian
University of New Mexico Health Sciences Center
Emily Alden
University of New Mexico Health Sciences Center
Erica Pascetti
University of New Mexico Health Sciences Center
Lily Y. Gu
University of Utah
Ali I. Saeed
Saint Joseph's Hospital and Medical Center

Research

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Abstract

Background: Cytokine profiles have traditionally been explored in serum due to its ease of accessibility and the diagnostic and assessment capabilities in a clinic setting. Utilization of additional cytokine depots, such as hilar lymph nodes, has not thoroughly been explored. In this study, we examined the cytokine profile of mediastinal and hilar lymph node fine needle aspirates to identify markers capable of differentiating high-risk smokers (>30 pack-years) from low-risk smokers (<30 pack-years), independent of current cancer diagnosis.

Methods: We used the cytokine profiles of 27 patients from a pro-spective convenience pilot study conducted at the University of New Mexico. Logistic regression analysis was employed.

Results: A significant difference in mean cytokine values for Leptin was discovered between patients categorized as low-risk and high-risk pack year smokers (p=0.034). Additionally, mean cytokine values of Leptin did not differ between patients by cancer diagnosis (malignant vs. benign). Our analysis demonstrated Leptin as a fair marker for discriminating between high-risk smokers and low-risk smokers (AUC 0.73).

Conclusions: We conclude Leptin is an optimal cytokine to discriminate between high-risk and low-risk smokers. To our knowledge, this is the first study to assess the ability of Leptin to serve as such an indicator via hilar lymph nodes.

Introduction

Smoking is the number one cause of preventable disease in the world and ac-counts for more than 25% of cancer deaths in the United States [1, 2]. It is associated with an overwhelming disease burden that includes coronary heart disease, stroke, chronic obstructive pulmonary disease, and lung cancer [3]. The weight of this behav-ior on healthcare systems has made the assessment of smoking history in patients an important research priority [4]. Current smoking assessment and monitoring ap-proaches range from self-reporting to body sensors that detect patterned movement. Self-reporting can only provide a rough estimate of smoking rates and does not allow for an assessment of smoke exposure levels [5]. Another approach is measuring the serum level of a nicotine metabolite called cotinine [6, 7]. Unfortunately, due to its rel-atively short half-life and incompatibility with nicotine patches, cotinine is a less than ideal solution.

A current area of exploration is serum cytokine profiling in smokers. Of note, it has been documented that cigarette smoking may exert contextual inflammatory modulation [8]. In patients with rheumatoid arthritis, an autoimmune disease that causes chronic inflammation of the joints, smoking status has been associated with the upregulation of inflammatory cytokines such as IL-2, IL-6, IL-12, IL-12p70, IFNγ, GM-CSF, MCP-1, and TNF-α within serum, suggesting cytokine profiling may be a more reliable assessment modality compared to tools that are currently available [9].

Endobronchial ultrasound bronchoscopy (EBUS) is now the standard of care for simultaneous diagnosis and staging of lung cancer. It allows for real-time fine needle aspiration of hilar lymph nodes and it was recently demonstrated that cytokine profil-ing of the aspirate tissue could discriminate between metastatic and benign hilar lymph nodes [10]. To the best of our knowledge, the relationship between cytokine pro-files in the hilar lymph node and smoking has never been addressed. In this study, we aimed to identify a cytokine profile from lymph node fine needle aspirate that is both sensitive and specific enough to reliably differentiate high-risk smokers from the pop-ulation, regardless of cancer diagnosis. We hypothesized that the cytokine profile of high-risk smokers would differ from the cytokine profile of low-risk patients, inde-pendent of cancer status.

Materials And Methods

Study Design

This is a prospective convenience pilot study conducted exclusively at the Uni-versity of New Mexico (University of New Mexico IRB: 16–363). IRB-approved patient consent was obtained for 28 patients and endobronchial ultrasound-guided fine needle aspiration biopsies were successfully conducted on 27 patients. One patient had the procedure terminated secondary to severe intraoperative hypoxia.

Sample collection, processing, and analysis

Sample and data collection procedures were conducted as previously described by Saeed et al. [10]. Briefly, our team utilized a convenience-sampling method to recruit patients from an outpatient lung clinic at the University of New Mexico Hospital. EBUS-guided fine needle aspiration biopsies were collected from mediastinal and hilar lymph nodes. Histologic diagnosis of lymph node aspiration biopsies was conducted by a staff pathologist at the University of New Mexico Hospital. Our team utilized a Bio-Rad Bioplex 200 suspension array system (Bio-Rad Laboratories, Inc., 171–000201) for cytokine analysis allowing for multiple protein analysis of individual samples. All samples were attempted to be processed simultaneously. We used Bio-Rad Bioplex Da-ta Pro software (Bio-Rad Laboratories, Inc., 1710001513) to identify extreme values, data distribution, and selection of range.

Statistical Analysis

SAS 9.4 (SAS Institute, Cary, NC) was used for statistical analysis. Data were nat-ural log-transformed, and variable distributions were described using mean and standard deviation. Smoking status was classified as low-risk (smoking history of less than 30 pack-years) or high-risk was (greater than or equal to 30 pack-years), a stand-ard set forth in the National Lung Screening Trial. The two-sample independent t-test was used to compare cytokine expression: 1) between high-risk smokers and low-risk smokers, 2) between benign and malignant cancer patients, and 3) between “any lung cancer diagnosis” and “any other cancer diagnosis” patients. Classification power was determined using univariate logistic regression analysis and was presented with an area under the receiver operating characteristic (ROC) curve (AUC). To identify opti-mal cutoffs for cytokines, we minimized the Euclidean distance, denoted by

D, between (0,1) and the ROC curve, using the following formula where Sn and Sp denote sensi-tivity and specificity respectively [11]:

$$D = \sqrt{(1 - \mathrm{Sn})^2 + (1 - \mathrm{Sp})^2} \qquad (1)$$

Results

Characteristics of Patient Cohort

The mean age of this cohort of 27 patients was 64.7 years old (SD: 9.46), with a range from 45 to 85 years old (Table 1). There was a higher frequency of males (70.37%) than females (29.63%). Patients had an average smoking history of 25.7 pack-years (SD: 20.3) with a range of 0 to 56 pack-years. The first quartile was at 3 pack-years and the third quartile was at 40 pack-years with an interquartile range of 37 pack-years. Low-risk smokers consisted of 12 patients (44.44%) with a mean age of 61.4 (SD: 10.14), and high-risk smokers consisted of 15 patients (55.56%) with a mean age of 67.4 (SD: 8.27) (Table 1).

Table 1
Descriptive statistics of the patient cohort by smoking status

	Smoking status (pack-years)		
	≥ 30	**< 30**	Total
	n[1] **(%)**[2]		
Gender			
Male	12	7	19 (70.37%)
Female	3	5	8 (29.63%)
Total	15 (55.56%)	12 (44.44%)	27 (100%)
Cancer status			
Benign	5	7	12 (44.44%)
Malignant	10	5	15 (55.56%)
Total	15 (55.56%)	12 (44.44%)	27 (100%)
Malignant cancer type			
Lung	6	3	9 (60.00%)
Other, non-lung cancer	4	2	6 (40.00%)
Total	10 (66.67%)	5 (33.33%)	15 (100%)
	Mean (Standard Deviation)		
Age (years**)**	67.4 (8.27)	61.4 (10.14)	64.7 (9.46)

[1]Frequency of patients. [2]Row or column percentage.

Biomarker Analysis

A significant difference between low-risk and high-risk smokers was found exclu-sively between the mean values of the cytokine Leptin ($p = 0.034$; Table 2). Namely, high-risk smokers had significantly downregulated Leptin cytokine values compared to low-risk smokers (Fig. 1). Additionally, IL-8 upregulation ($p = 0.0663$) and VEGF-A upregulation ($p = 0.0986$) were on the boundary of significance. Mean cytokine values of Leptin did not significantly differ between patients with malignant and benign cancer diagnoses. Both IL-8 ($p = 0.0011$) and VEGF-A ($p < 0.0001$) demonstrated a significant difference between mean cytokine values. A secondary analysis comparing mean cyto-kine values of Leptin between patients with “any lung cancer diagnosis” and patients with “any other cancer diagnosis” showed no significant difference.

Table 2
Mean cytokine values (pg/mL) by smoking status.

Cytokine	< 30 pack-years	≥ 30 pack-years	*p*-value
Leptin	7.94	7.80	0.0342
IL-8	4.87	6.18	0.0663
VEGF-A	7.10	8.21	0.0986
Angiopoietin 2	6.54	7.12	0.1031
IL-18	5.67	5.21	0.1476
SCF	5.64	5.27	0.2022
IL-6	3.78	4.48	0.2239
PLGF	4.07	4.56	0.2494
sIL-6Ra	9.22	8.73	0.2778
Osteopontin	9.28	9.87	0.321
VEGF-D	5.51	5.72	0.327
sVEGFR 1	7.53	8.05	0.3356
sVEGFR 2	8.11	7.84	0.3365
TGF a	3.90	4.20	0.3407
Endoglin	6.66	6.46	0.3425
Follistatin	6.89	7.24	0.4191
G CSF	4.97	4.61	0.4381
sEGFR	9.20	8.95	0.4393
sTIE 2	8.14	7.96	0.4772
PDGF AB BB	6.52	6.29	0.4813
TNF a	2.59	2.43	0.5252
sCD40L	5.60	5.78	0.5288
HB EGF	4.14	4.36	0.5565
PECAM	8.25	8.14	0.5776
sHER 2neu	8.61	8.68	0.6853
sFASL	5.14	5.21	0.731
uPA	6.08	5.83	0.7336

Cytokine	< 30 pack-years	≥ 30 pack-years	*p*-value
IGFBP 1	7.92	8.02	0.8182
PAI 1	10.13	10.22	0.8282
HGF	8.46	8.39	0.8645
FGF basic	6.56	6.50	0.8648
Prolactin	8.59	8.55	0.9243
VEGF-C	5.76	5.76	0.9954
EGF	4.60	4.60	0.998

Logistic regression analysis demonstrated Leptin to be a fair marker for discriminating between high-risk and low-risk smokers (AUC of 0.7083) (Fig. 2). For screening and diagnostic purposes, the ideal cutoff for our cytokine is a natural log-transformed value of 7.283. When this cutoff was applied to our sample, we demonstrated a sensitivity of 67%, specificity of 75%, positive predictive value of 77%, and negative predictive value of 64%, assuming sample prevalence, at identifying high-risk smokers from low-risk smokers.

Discussion

Exposure to cigarette smoke components can induce an immune response thought to contribute to cytokine concentration variability. Previous studies have explored cy-tokine changes following smoking cessation and have found decreased inflammatory cytokine titers in individuals who have eliminated smoking activity. Brenner et al. demonstrated that current smoking status was associated with several inflammation markers. They observed an increase in IL-6 and IL-8 expression had an increased risk for lung cancer and proposed that IL-6 and IL-8 promote tumorigenesis by acting on lung epithelial cells when signaling through the nuclear factor pathway [12].

Cotinine is one of the most common biomarkers used to validate patient-reported smoking status and has been well utilized since the late 1990s [6, 7]. Cotinine is found in tobacco and is a metabolite of nicotine with a half-life of approximately 17 hours [13]. Unfortunately, the specificity for tobacco use drops for persons using nico-tine-containing medication. Furthermore, although cotinine provides information re-garding smoking activity proximal to sample analysis, chronic behavior and disease risk are not addressed through this sampling modality.

Findings from this current study suggest Leptin is a potential biomarker for smoking categorization of patients. There are multiple studies implicating the rela-tionship between Leptin and smoking, but to the

best of our knowledge, ours is the first study assessing the efficacy of Leptin as a biomarker for smoking status from lymph node fine needle aspirates. Previous studies have demonstrated smoking cessation results in increased Leptin and Leptin could be a significant contributor to the correla-tion between smoking and body mass index (BMI) [14–16]. It has been suggested that elevated plasma Leptin following smoking cessation may be due to either increased Leptin secretion from adipose tissue or decreased removal of Leptin. However, data implicating Leptin in smoking has been somewhat inconsistent. It has been shown that cigarette smoking increases the release of glucocorticoids from the adrenal glands [17]. This elevation in glucocorticoids has been shown to increase Leptin expression within adipose tissue and paints an inconsistent picture of the correlation between smoking and Leptin levels. In our study, patients with a $\geq$ 30 pack-year smoking history had a statistically significant decrease in Leptin expression compared to low-risk smokers, suggesting that smoking results in a cumulative expression change over time as op-posed to an immediate response to carcinogens or nicotine. Interestingly, increased serum levels of leptin are associated with greater craving and difficulty in achieving abstinence from smoking [18]. The utilization of Leptin to assess current smoking pat-terns and disease risk may prove to be advantageous for physicians and patients when tackling smoking patterns or secondary disease sequalae.

Study limitations that could affect data interpretation include our small sample size, which may have weakened the statistical power of our analyses. However, we met all requirements for a pilot study, and we were able to demonstrate the selective predictive ability of Leptin despite this potential shortcoming. We assumed that cyto-kine profiles associated with metastases would be the same across cancer types and thus, our sample included different cancer types. We do not anticipate that changes in this assumption would influence the results. An adjustment for multiple comparisons was not done as (a) our analysis was meant to be exploratory and (b) most results of this study were not significant and thus any obscuration of true associations was avoided.

Smoking has short-term and long-term effects on both individual and population health. It is accepted that the only way to reduce cancer risk in patients who smoke is complete smoking cessation. Since patients may be unable or unwilling to quit, utiliza-tion of biomarker panels by physicians may provide an effective means of assessing a patient's current smoking patterns and cancer risk in real time. Furthermore, in-traoperative diagnostics utilizing Leptin may provide information regarding disease origin and thus, contribute to the development of targeted therapies.

Conclusions

This study sought to contribute to our understanding of the relationship between cytokines and smoking within hilar lymph nodes. We present, for the first time, a po-tential optimal cytokine cutoff to discriminate between high-risk smokers and low-risk smokers within mediastinal hilar lymph nodes. While this research provides the op-portunity to categorize disease status with smoking status to better inform both disease treatment and patient management, further studies with larger sample sizes need to be taken up to establish Leptin as an affirmative biomarker of high-risk smoking.

Declarations

Ethics approval and consent to participate:

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (IRB) of the University of New Mexico Health Sciences (IRB#: 16-363). Informed consent was obtained from all subjects involved in the study.

Consent for publication:

All authors have read and approved the content, and agree to submit it for consideration for publication in your journal.

Availability of data and materials:

The data presented in this study are available on request from the corresponding author.

Competing interests:

The authors declare that they have no competing interests

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Author Contributions:

Conceptualization, F.Q. and A.I.S.; methodology, R.A., E.A., F.Q., and A.I.S.; software, R.A., E.A., F.Q., and A.I.S.; validation, R.A., E.A., L.Y.G., F.Q., and A.I.S.; formal analysis, R.A., E.A., L.Y.G., and F.Q.; investigation, R.A., E.A., F.Q., and A.I.S.; resources, R.A., E.A., F.Q., and A.I.S.; data curation, R.A., E.A., F.Q., and A.I.S.; writing—original draft prepara-tion, R.A., E.A., E.P., F.Q., and A.I.S.; writing—review and editing, R.A., E.A., E.P., L.Y.G., F.Q., and A.I.S.; visualization, F.Q. and A.I.S.; supervision, F.Q.; project administration, F.Q.; funding acquisition, A.I.S. All authors have read and agreed to the published version of the manuscript.

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Figures

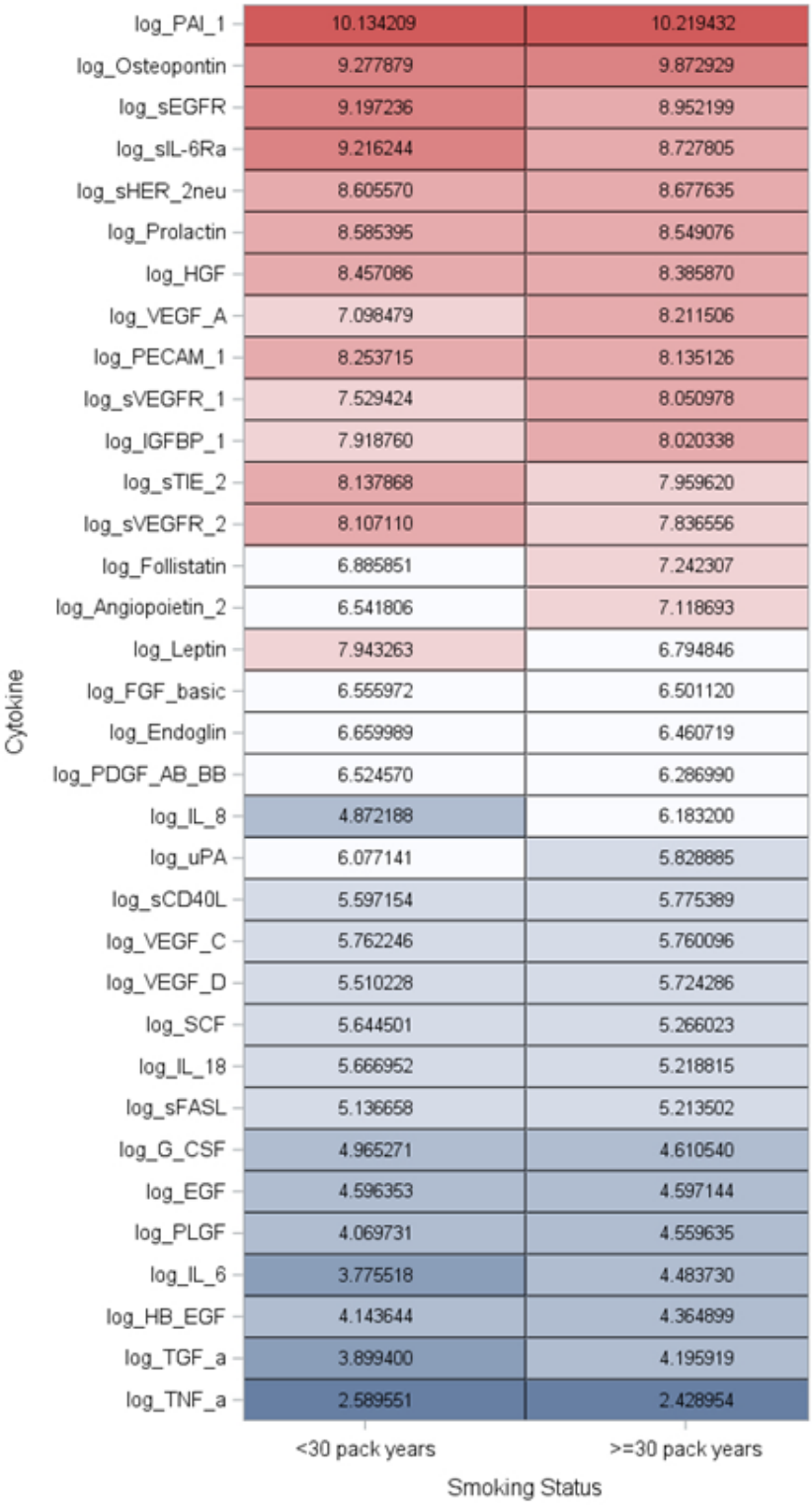

Figure 1

Distribution heat map of mean cytokine values (pg/mL) by smoking status.

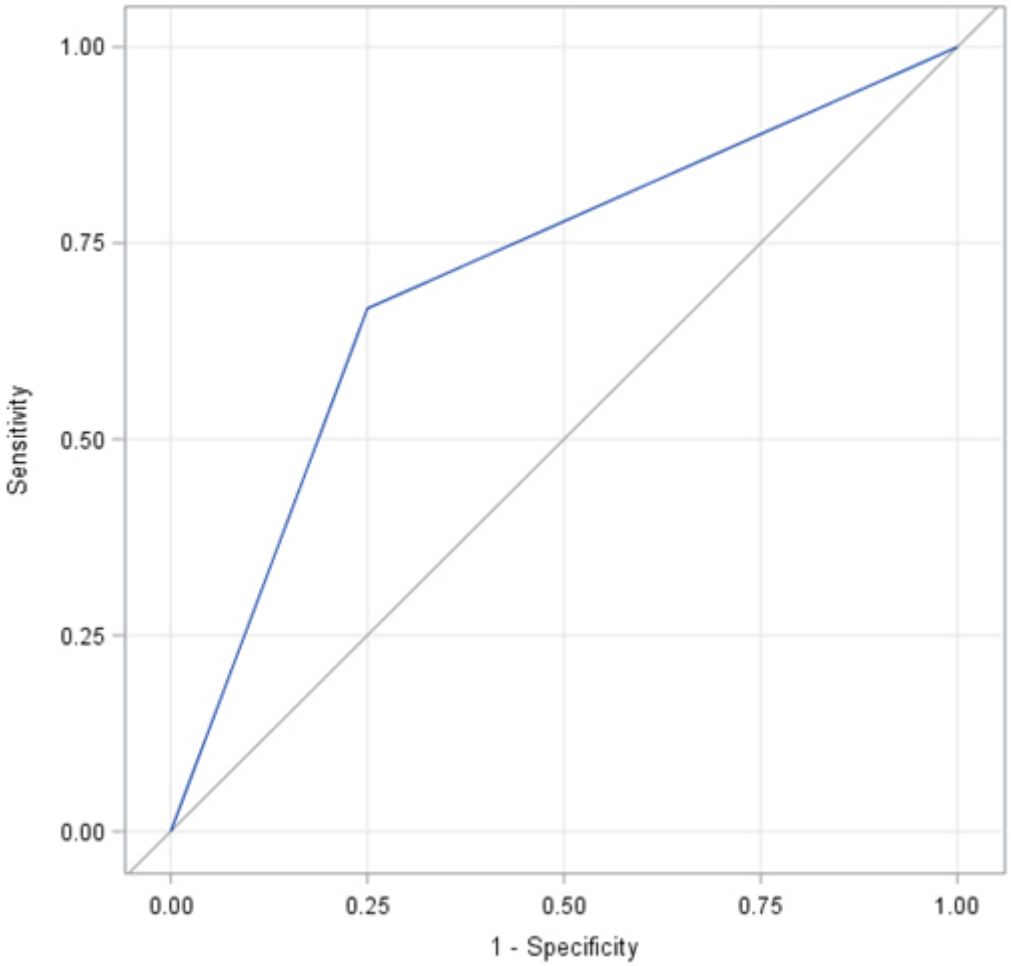

Figure 2

Receiver operating curve (ROC) curve of the logistic regression model using Leptin as a binary variable (a natural log-transformed value of 7.283 as a cutoff) to predict smoking status (AUC 0.7083).